

JNK Isoforms Differentially Regulate Neurite Growth and Regeneration in Dopaminergic Neurons In Vitro

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Abstract Parkinson's disease is characterized by selective and progressive loss of midbrain DAergic neurons (MDN) in the substantia nigra and degeneration of its nigrostriatal projections. Whereas the cellular pathophysiology has been closely linked to an activation of c-Jun N-terminal kinases (JNKs) and c-Jun, the involvement of JNKs in regenerative processes of the nigrostriatal pathway is controversially discussed. In our study, we utilized a mechanical scratch lesion paradigm of midbrain DAergic neurons in vitro and studied regenerative neuritic outgrowth. After a siRNA-mediated knockdown of each of the three JNK isoforms, we found that JNKs differentially regulate neurite regeneration. Knockdown of JNK3 resulted in the most prominent neurite outgrowth impairment. This effect was attenuated again by plasmid overexpression of JNK3. We also evaluated cell survival of the affected neurons at the scratch border. JNK3 was found to be also relevant for survival of MDN which were lesioned by the scratch. Our data suggest that JNK isoforms are involved in differential regulation of

cell death and regeneration in MDN depending on their neurite integrity. JNK3 appears to be required for regeneration and survival in the case of an environment permissive for regeneration. Future therapeutic approaches for the DAergic system may thus require isoform specific targeting of these kinases.

Keywords JNK3 · Dopaminergic (DAergic) neuron · Regeneration · Parkinson's disease

Introduction

Parkinson's disease (PD) is the second most common aging-related neurodegenerative disorder and is characterized by progressive loss of dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNpc) of the midbrain (Olanow and Tatton 1999). Several gene mutations have been linked to familial PD (Farrer 2006) but the etiology of sporadic PD, which accounts for 90% to 95% of all PD cases, is largely undefined. Current hypotheses attribute an increased risk for sporadic PD to misfolding and aggregation of proteins, mitochondrial dysfunction and oxidative stress. At the time of PD diagnosis, about 80% of DAergic neuron terminals in the striatum and 60% of DAergic neurons in the SNpc are already lost in PD brains (Dauer and Przedborski 2003). Today's medical therapies can only focus on symptomatic relief, and there is a great need to identify new therapeutic targets for improved clinical performance. As studies aiming to protect DAergic neurons in the SNpc have largely failed (Olanow et al. 2008; Lohle and Reichmann 2010) there is increasing interest in DAergic axonal targets which most likely play an earlier role in disease pathology (Braak et al. 2003; Duda 2003; Conforti et al. 2007;

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Schulz-Schaeffer 2010) and may be the substrate of regenerative approaches. For example, functional enhancement and protection of DAergic terminals could significantly improve clinical symptoms in animal models of Parkinson's disease (Chung et al. 2009).

The c-Jun N-terminal kinases (JNKs) are commonly known as stress-activated protein kinases because their activities typically increase in response to different cellular environmental stresses like osmotic stress, redox stress, or irradiation (Davis 2000; Weston and Davis 2002). Once activated by a stress response or by inflammatory cytokines they can also mediate cellular apoptosis (Kyriakis and Avruch 1996; Ip and Davis 1998) - a function that is particularly prominent in neurons and neuron-like cells (Xia et al. 1995; Watson et al. 1998).

Increasing in vitro and in vivo evidence suggests a role for JNK in the pathogenesis of DAergic neuron death in several Parkinson's disease models (Peng and Andersen 2003). As one example, the JNK pathway including its upstream kinase MKK4 is activated in the nigrostriatal system after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) application in mice (Saporito et al. 2000; Willesen et al. 2002). Consequently, CEP-1347, a pharmacological inhibitor of upstream kinases of JNK, was able to protect from MPTP-induced JNK activation and nigrostriatal DAergic neuron loss (Saporito et al. 1999). These data are confirmed by later studies that in addition looked more closely at destruction of axonal TH-positive DAergic terminals in the striatum. Together with a protracted expression and phosphorylation of c-Jun in the SNpc after median forebrain bundle lesion Crocker et al. found a pronounced loss of TH-positive terminals in the striatum. Dominant-negative c-Jun did not only inhibit axotomy-induced dopamine neuron death and attenuate phosphorylation of c-Jun in nigral neurons but also delayed the degeneration of TH-positive DAergic terminals in the striatum (Crocker et al. 2001). Systemic application of the mixed lineage kinase inhibitor CEP11004 in the 6-hydroxydopamine model of Parkinson's disease inhibited the loss of DA neurons in the SNpc and preserve TH-positive striatal termini, too (Ganguly et al. 2004).

Whereas the aforementioned studies manipulated the entire JNK pathway (mainly due to the lack of specific pharmacological inhibitors) there is evidence that the three mammalian

JNK proteins (Gupta et al. 1996) play different roles in the context of cell survival and axon regeneration. Neuronal cell stress was found to specifically activate only JNK2 and 3 (Coffey et al. 2002). The function of these two genes seems to be closely related: in mice with single or double *jnk2* and *jnk3* null mutations only the double null mutation could protect from apoptosis in the intrastriatal 6-hydroxydopamine neurotoxin model (Ries et al. 2008). These results are in opposite to the lack of protection in the axonal compartment, where rather intense axonal degeneration in the *jnk2* and *jnk3* null mutations was observed. Similarly, the upstream blockade of the JNK pathway in the same animal model using an adeno-associated virus vector delivery of dominant-negative forms of dual leucine zipper kinase strongly inhibited apoptosis and enhanced long-term survival of DAergic neurons but did not protect their axons (Chen et al. 2008).

In order to further clarify the role of the three JNK proteins for axonal regeneration in DAergic neurons we performed a study of differential siRNA-mediated knock-down of JNK isoforms and evaluated neurite regeneration and DAergic survival in the scratch paradigm of mechanically transected primary neurons in culture (Knöferle et al. 2010). We identify JNK3 as the most important isoform regulating neurite outgrowth and survival after lesion.

Materials and Methods

siRNAs and Plasmids

siRNA targeting rat JNK1, JNK2, JNK3 and EGFP (GFP-22 siRNA) were purchased from Qiagen (Hilden, Germany). siRNA sequences are provided in Table 1.

Primary Midbrain Neuron Culture

Primary midbrain DAergic cultures were prepared according to previously published protocols (Knöferle et al. 2010). Briefly, the mesencephalon floor of embryonic day 14 Wistar rats was dissected and the meninges were removed. The dissected tissue pieces were collected in ice-cold CMF and centrifuged at 1,000 rpm for 4 min. Trypsin (750 µl,

Table 1 Sequences for JNK1, JNK2, JNK3, and EGFP siRNA

	siRNA sequence	Accession number	Target sequence (bp)
JNK1 siRNA	5' AGGCGAGAGATTGTTATCTA 3'	XM_341399	1,003–1,023
JNK2 siRNA	5' CTGGTGAAAGGTTGTGTGATA 3'	NM_017322	668–688
JNK3 siRNA	5' TCCGTATGTGGTGACGAGATA 3'	NM_012806	912–932
EGFP siRNA	5' CGGCAAGCTGACCCTGAAGTTCAT 3'	–	–

0.25%, Sigma) was added to the tissue pellet and after 15 min of incubation at 37°C was inactivated with 750 µl cold FCS. Tissue fragments were gently triturated, the cell suspension was centrifuged at 1,000 rpm for 4 min and resuspended in culture medium.

For RNA interference studies, 4×10^6 cells were transfected with 0.3 µg siRNA and/or 0.5 µg plasmidic DNA using Amaxa Nucleofector (Amaxa, Cologne, Germany). Cells were then plated at a density of 500,000/well on 24-well plates (Sarstedt, Nümbrecht, Germany) containing coverslips coated with poly-D-lysine and laminin.

For JNK inhibition studies, cells were plated at a density of 500,000/well on 24-well plates (Sarstedt) directly after dissection. From day 3, cells were incubated with 5 µM of the small molecule ATP-competitive JNK inhibitor SP600125 (anthra(1,9-cd)pyrazol-6(2 H)-one; CalbioChem, Darmstadt, Germany) or DMSO (AppliChem, Darmstadt, Germany) for 3 days before lysis/fixation (Bennett et al. 2001).

Cell cultures were maintained at 37°C in a 5% CO₂ humidified atmosphere in DMEM-F12 (Invitrogen) supplemented with 2.5 mg/ml BSA (35%), 0.9% D-(+)-glucose solution (45%), 2 mM L-glutamine (PAA Laboratories, Pasching, Austria), 5 µg/ml insulin, 1:100 N1 medium supplement and 1:100 PSN antibiotic mixture (Invitrogen, Scotland, UK) for 4, 7, or 9 days. Medium was changed 24 h after cell dissection and subsequently every second day.

Scratch Assay and Phase Contrast Imaging

Three days following cell plating, cells were submitted to mechanical transection using a self-made 2 mm broad silicon rubber scratch device. Each coverslip was microscopically examined to ensure completeness of the scratch. Three days after mechanical transection (on day 6), cells were incubated in a climate chamber for live cell imaging (37°C, 5% CO₂) on a fluorescence inverted microscope (Axiovert, Zeiss, Oberkochen, Germany) equipped with a CCD camera and AxioVision software (Zeiss, Göttingen, Germany). Contrast phase photographs of three random visual fields per culture well were taken with a 20× objective.

Immunocytochemistry

For DAergic cell visualization, cells were fixed after live imaging on day 6 in PFA 4% 10 min at room temperature (RT, 22°C), permeabilized with 100% ice-cold acetone (AppliChem) 10 min at −20°C, washed twice with PBS and blocked with 10% normalized goat serum 10 min at RT. Probes were incubated with the primary antibodies (rat anti-tyrosine hydroxylase 1:250 (Advanced Immunochemicals, Long Beach, CA, USA) overnight at 4°C. Following 2 PBS washes, appropriate Cy3- or Cy2-labeled secondary antibodies (1:250, Dianova, Hamburg, Germany) were applied

for 45 min at 37°C. Cells were then nuclear counter-stained with DAPI (4,6-diamidino-2-phenylindole) (Sigma, Taufkirchen, Germany) and mounted in Mowiol (Hoechst, Frankfurt, Germany). Fluorescence was observed and recorded on a Zeiss Axioplan 2 fluorescence microscope equipped with a CCD camera and AxioVision software (Zeiss). For evaluation of DAergic regeneration and survival at the scratch site, pictures of the entire scratch site were taken with a 20× objective. For evaluation of DAergic survival within the culture, pictures of two random visual fields per culture well were taken with a 20× objective.

Quantification of Regenerating Fibers

Neurite length was evaluated semi-automatically using the axon tracing module of “Image J” (Free Java software provided by the National Institutes of Health, Bethesda, Maryland, USA). For evaluation of whole culture regeneration, the length of the 20 longest axons extending from the scratch site of each photomicrograph was quantified. Neurite length was defined as the neurite extension from neurite tip to scratch site. For evaluation of DAergic regeneration, length of all TH-labeled axons extending from the scratch site of each photomicrograph was quantified. The DAPI-labeled edge was used to identify the scratch site. Results are expressed in % of EGFP-transfected cells. Experiments were performed at least in triplicate.

Quantification of DAergic Neuron Survival

For survival of DAergic neurons at the scratch site, the number of TH-labeled cells located in the area comprising the first 50 µm next to the scratch site was quantified.

Statistics

All in vitro experiments were reproduced at least in triplicate. Differences between groups were considered statistically significant according to a one-way ANOVA followed by a parametric multiple comparison test (Dunnett’s test). Significances were indicated with * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, unless otherwise stated.

Results

Pharmacological Inhibition of All JNK Isoforms (1, 2, and 3) Impairs Neurite Regeneration of Primary DAergic Neurons

In order to evaluate the effect of a combined inhibition of all three JNK isoforms we took advantage of the

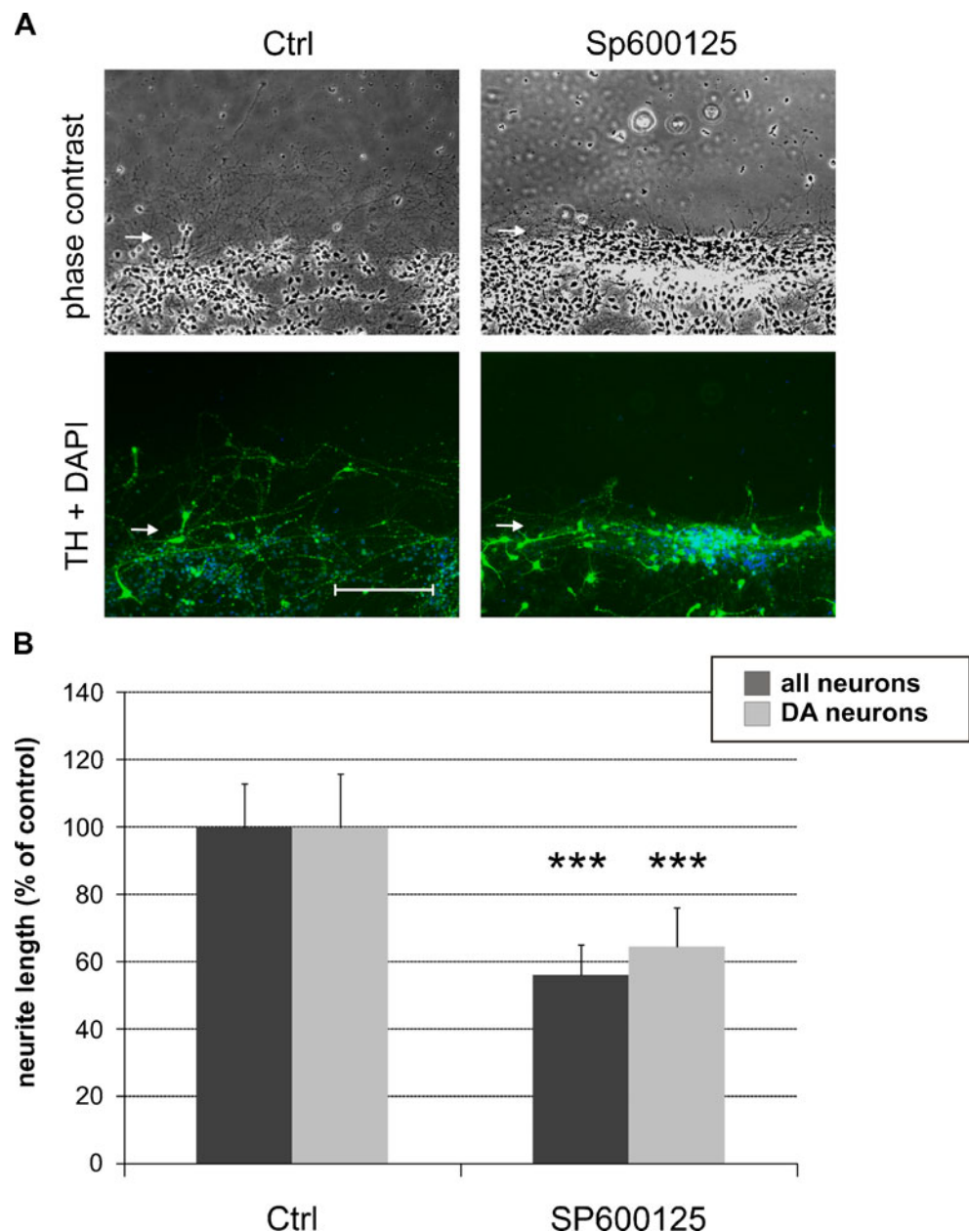
availability of the pharmacological small molecule ATP-competitive pan-JNK inhibitor SP600125. Primary DAergic neurons were cultured under standard conditions. After 3 days neurites were mechanically transected and SP600125 was applied at the same time. The evaluation of neurite outgrowth of DAergic neurons from the scratch border after another 3 days showed a significantly decreased relative outgrowth of JNK inhibitor treated total neurons ($56.2 \pm 8.8\%$) in comparison to non-inhibitor treated cells that were set to 100% ($100 \pm 13.0\%$) (Fig. 1). The same holds true for a specific analysis of only DAergic neurons that show a decreased outgrowth after JNK inhibitor treatment ($64.4 \pm 32.8\%$) in comparison to

untreated DAergic neurons ($100 \pm 15.9\%$). There was no significant difference between regeneration of DAergic and non-DAergic neurons.

siRNA-Mediated Downregulation of JNK3 Most Significantly Impairs Regenerative Neurite Outgrowth in Primary MDN

Midbrain DAergic neurons (MDN) cultures were separately transfected with siRNAs directed against JNK1, 2, and 3. After 3 days in vitro, the neurons were scratch lesioned and their regenerative neurite outgrowth was evaluated after another 3 days (Fig. 2). In comparison to the siEGFP

Fig. 1 SP600125 significantly inhibits regenerative neurite outgrowth. **a** Representative micrographs of MDN cultures at 3 days after scratch lesion (*upper panel*, phase contrast; *lower panel*, TH-Cy2 (green) and DAPI (blue)). Cultures were treated with the pan-JNK-inhibitor SP600125 (5 μ M) or DMSO control. *Arrow*, site of mechanical transection (scratch lesion). *Scale bar*, 150 μ m. **b** Treatment with the pan-JNK-inhibitor SP600125 (5 μ M) significantly impairs neurite regeneration of all primary cultured neurons including TH-positive DAergic subpopulation after mechanical transection. Data are from three independent experiments each. *Bars* represent means \pm SEM; *** $p < 0.001$



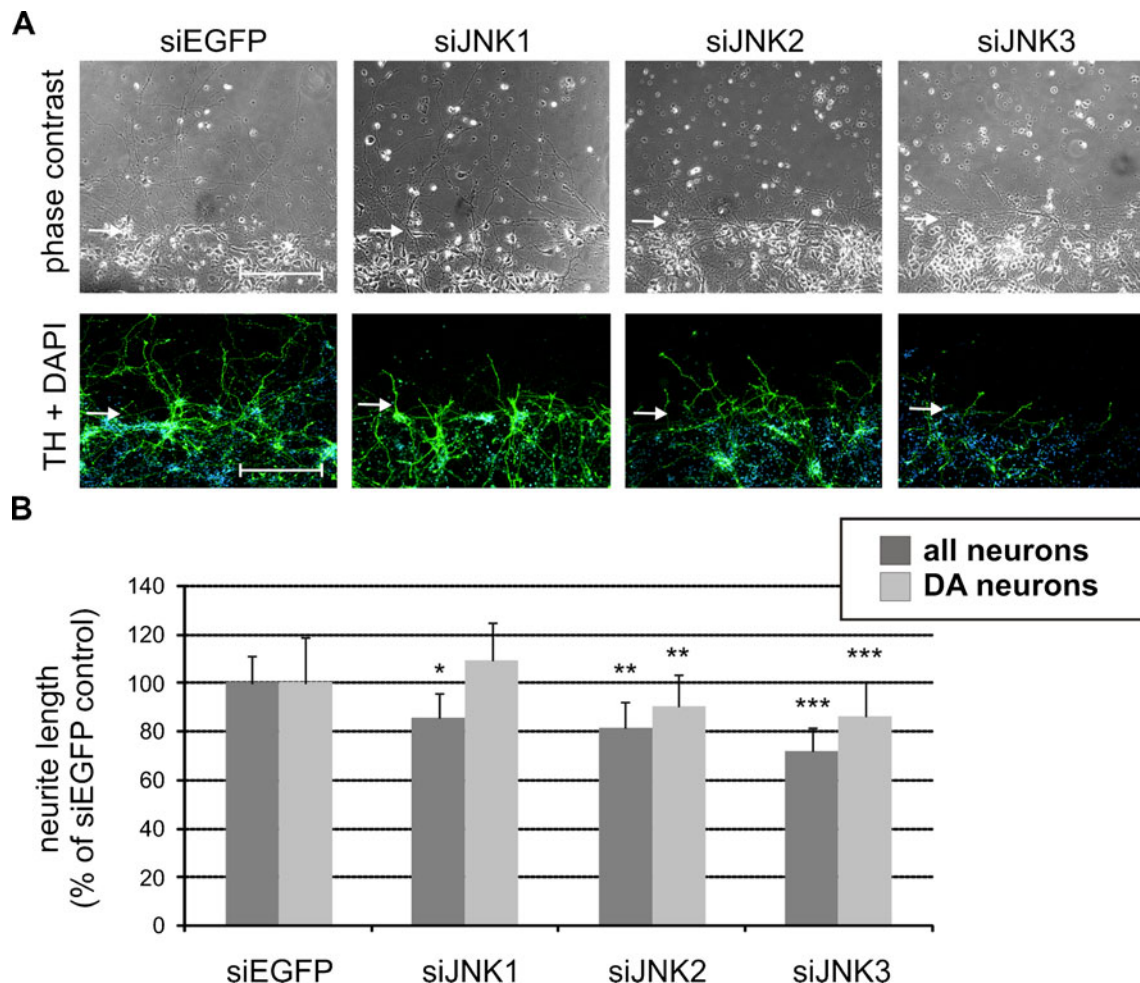


Fig. 2 Downregulation of JNK isoforms negatively influences regenerative neurite outgrowth. **a** Representative micrographs of MDN cultures at 3 days after scratch lesion (*upper panel*, phase contrast; *lower panel*, TH-Cy2 (green) and DAPI (blue)). Cultures were transfected with 0.3 µg of siRNA directed against EGFP, JNK1, JNK2, or JNK3, respectively. Arrow, site of mechanical transection (scratch lesion). Scale bar, 200 µm. **b** Quantitative analysis of neurite

length at 3 days after mechanical transection in MDN cultures treated with different siRNAs for all neurons and for the DAergic subpopulation only. Application of JNK3 siRNA (*siJNK3*, 0.3 µg) resulted in the most significant decrease in neurite regeneration compared with cells transfected with EGFP siRNA (*siEGFP*) as a control. Bars represent means ± SEM. For all treatments, $n=8$. * $p<0.05$; ** $p<0.01$; *** $p<0.001$

control ($100\pm11.4\%$), siJNK1 only slightly decreased neurite outgrowth in the total population ($85.6\pm10.2\%$). This effect was more pronounced with siJNK2, which reduced neurite outgrowth in the total population to $81.5\pm10.7\%$ and in DAergic neurons to $90.3\pm13.1\%$. Most significantly, knockdown of JNK3 reduced neurite outgrowth to $72.0\pm9.7\%$ in the total population and to $86.2\pm14.4\%$ in the DAergic neuron subpopulation.

siJNK3-Related Impaired Regenerative Response Can Be Efficiently Rescued by Overexpression of JNK3

As the neurite regenerative outgrowth was most dependent on the JNK3 isoform (Fig. 2), we overexpressed the JNK3 protein in culture to evaluate the specificity of our

approach (Fig. 3). SiJNK3 transfection of MDN cultures resulted in a significantly impaired neurite outgrowth after scratch lesion compared with siEGFP transfection in the total population ($66.4\pm11.2\%$ to $100.0\pm10.5\%$) and in the DAergic subpopulation ($70.5\pm11.9\%$ to $100.0\pm16.9\%$) three days after lesioning. Expression of pEGFP (total population, $93.3\pm12.1\%$; DAergic population, $122.8\pm22.5\%$) or pJNK3 alone (total population, $101.8\pm13.7\%$; DAergic population, $110.4\pm22.5\%$) did not significantly alter neurite outgrowth. However, the combined transfection of siJNK3 with pJNK3 significantly rescued the siJNK3 mediated impairment of neurite regeneration (total population, $94.5\pm13.8\%$; DAergic population, $99.9\pm16.9\%$). This effect could not be rescued by combined transfection of siJNK3 and pEGFP (total population, $74.7\pm12.5\%$; DAergic population, $87.8\pm15.7\%$).

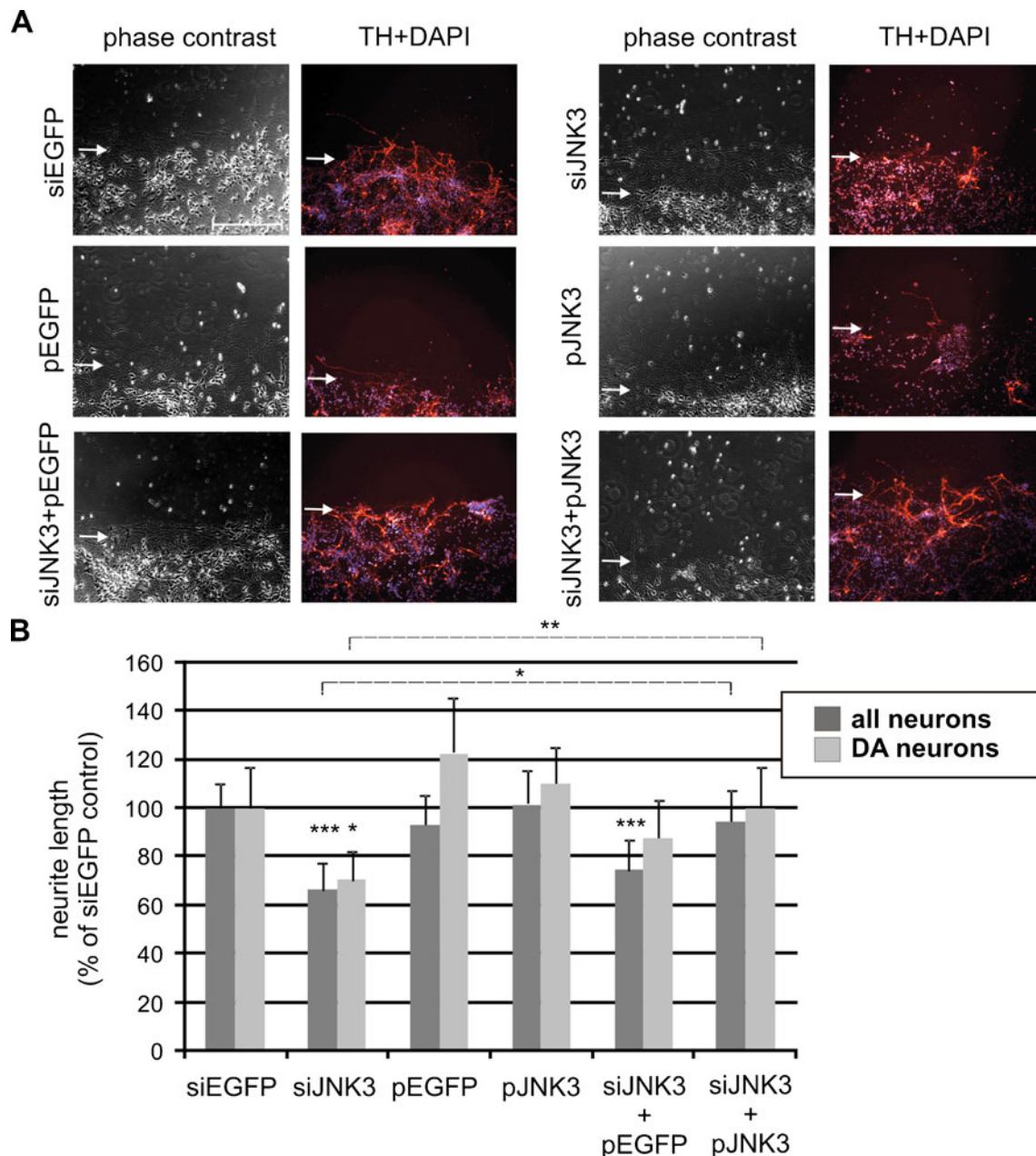


Fig. 3 JNK3 overexpression efficiently rescues the impaired regenerative response following JNK3 siRNA silencing. **a** Representative micrographs of MDN cultures at 3 days after scratch lesion (left panel, phase contrast; right panel, TH-Cy3 (red) and DAPI (blue)). Cultures were transfected on DIV0 with siRNA against EGFP or JNK3, the plasmids pEGFP or pJNK3, or a combination of JNK3 siRNA and pEGFP or JNK3 siRNA and pJNK3. Arrow, site of scratch lesion. Scale bar, 200 μ m. **b** Quantitative analysis of neurite length at 3 days

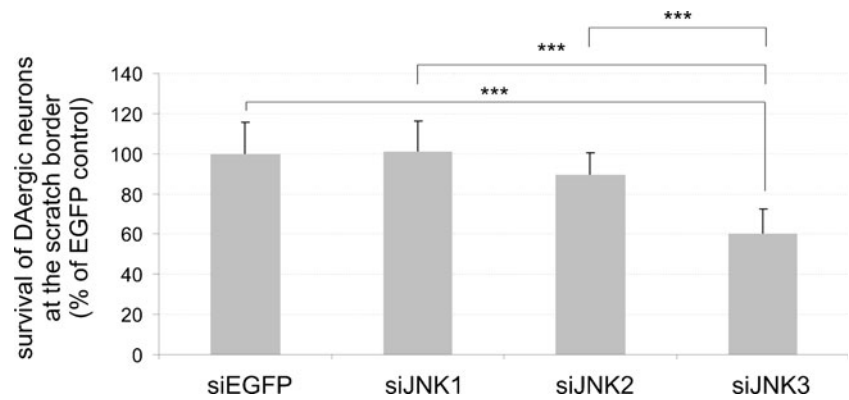
after scratch lesion in MDN cultures treated with siRNA or plasmid DNA as indicated. Neurite length is given for all neurons and for the DAergic subpopulation only. Overexpression of JNK3 rescues the siJNK3-mediated regeneration impairment, while transfection with pEGFP does not have a significant effect on neurite regeneration. Data are from three independent experiments each. Bars represent means \pm SEM. For all treatments, $n=8$. * $p<0.05$; ** $p<0.01$; *** $p<0.001$

Downregulation of JNK3 in Scratch Lesioned DAergic Neurons Impairs Survival

Apart from their role in neurite regeneration JNK proteins play an important function in cell survival. Therefore, we evaluated the number of surviving TH-

positive neurons at the scratch border after 6 days in culture and siRNA transfection at DIV0 (Fig. 4). siJNK3 transfected neurons demonstrated a significantly reduced survival ($60.3 \pm 11.9\%$) compared with the siEGFP ($100.0 \pm 15.5\%$) as well as to the siJNK1 ($101.0 \pm 15.4\%$) and siJNK2 ($89.8 \pm 10.8\%$) transfected neurons.

Fig. 4 JNK3 is important for survival of mechanically lesioned DAergic neurons. Transfection of 0.3 μ g of JNK3 siRNA significantly decreases the number of surviving DAergic neurons at the scratch border compared with EGFP-, JNK1- and JNK2-siRNA-transfected neurons. For all treatments, $n=8$. Experiments were repeated at least three times with cells from different cultures. Bars represent means \pm SEM. *** $p<0.001$



Discussion

In search for putative targets for neurorestorative strategies in Parkinson's disease (PD), we studied the role of JNK protein isoforms as they have been shown to participate in the pathophysiology of DAergic degeneration (Peng and Andersen 2003). We report here that the three JNK isoforms play distinct roles for neurite regeneration of DAergic neurons and that JNK3 seems to be the most relevant isoform in the regeneration paradigm after mechanical neurite lesion.

Pharmacological Inhibition of JNK Negatively Affects Neurite Regeneration

In the lesioned CNS, c-Jun phosphorylation occurs in axotomized neurons and correlates with enhanced long-term JNK activity (Herdegen et al. 1998). JNKs are able to trigger both expression and activation of c-Jun in lesioned neurons (Lindwall and Kanje 2005). However, several in vitro studies have shown that JNKs are also involved in neurite formation and stabilization (Leppa et al. 1998; Xiao and Liu 2003).

In our study, pan-JNK inhibition with SP600125 led to a severe impairment of neurite regeneration after scratch lesion in all neurons including the DAergic subpopulation (Fig. 1), which has been previously also reported for unlesioned MN9D cells and primary DAergic neurons (Eom et al. 2005). Thus, neurite growth in DAergic neurons depends on JNK activity in both primary and regenerative growth. Using RNAi technology we further investigated the question, which JNK isoform specifically regulates survival and regeneration in primary DAergic neurons.

Differential Role of JNK1, 2, and 3 for Neurite Regeneration in Midbrain Neurons

In a previous study, we could demonstrate the importance of distinct protein kinase families for growth cone collapse, neurite retraction and neurite outgrowth (Loh et al. 2008).

We now show that the downregulation of all three JNK isoforms regulates the regenerative response in the total population, but only downregulation of JNK2 and even more JNK3 had significant effects on the DAergic subpopulation. Plasmid-mediated overexpression of JNK3 reversed the effect of single-time siRNA application and thus confirmed the importance of JNK3 in regenerative signaling. Interestingly, overexpression of JNK3 alone did not result in an increased regenerative growth, suggesting that JNK3 signaling is already saturated in this context.

JNK1 and JNK2 were previously shown to be involved in neurite outgrowth in PC12 cells (Waetzig and Herdegen 2003b). Whereas JNK1 and JNK2 are expressed in a large variety of tissues, the JNK3 isoform is restricted to brain, heart, and testis (Gupta et al. 1996). Although JNK3 was mainly attributed a pro-apoptotic function (Yang et al. 1997), it was also able to enhance NGF-induced sprouting in PC12 cells that normally do not express JNK3 (Waetzig and Herdegen 2003a). A putative explanation for JNK3-associated neurite growth may be the fact that JNK3 together with JNK2 could be translocated to the nucleus and thereby influence transcription of regeneration-associated genes like c-Jun, ATF-3 (Lindwall et al. 2004), as well as CD44, galanin and α 7b1 integrin (Raivich et al. 2004; Raivich and Makwana 2007). Depending on cell type and cellular context JNKs can bind and activate different binding partners in so-called signalosomes and thus, in principle, can trigger both degeneration and neurite outgrowth (Waetzig and Herdegen 2005; Waetzig et al. 2006). For example, in an analysis using knockout mice for *jnk1*, *jnk2*, or *jnk3*, all animals presented a failure in neuritogenesis of hippocampal neurons, most prominently in the *jnk3* knockout. However, this effect was not observed in cortical neurons (Eminel et al. 2008). Even more conflicting was the finding in cerebellar neurons, where JNKs relatively inhibited neuritogenesis (Coffey et al. 2000). Very recently, a study analyzing axonal regeneration in adult mouse dorsal root ganglion neurons demonstrated that neuritogenesis is delayed by lack of JNK2 and JNK3 but not by JNK1. JNK1 and JNK2 were rather necessary

for sustained neurite elongation that was associated with the phosphorylation state of microtubule-associated protein MAP1b (Barnat et al. 2010). Our data support the notion that JNK3 is most prominently involved in regulation of the initial regenerative response in primary DAergic midbrain neurons.

JNK3 Is Relevant for Survival of Mechanically Lesioned DAergic Neurons

Our analysis demonstrated that JNK3-knockdown negatively affects survival of lesioned DAergic neurons at the scratch border (Fig. 4). Importantly, no general cell stressor was applied in this paradigm which may be the reason for the lack of detrimental signaling of JNK3. On the contrary, the regenerating and previously lesioned neurons seemed to partially depend on JNK3 since a downregulation resulted in less survival.

JNK3 appears to function as a key player in apoptosis (Waetzig and Herdegen 2003a). For example, *jnk3* knockout mice lack excitotoxicity-induced apoptosis in the hippocampus (Yang et al. 1997). Targeted deletion of *jnk3* did not only reduce the stress-induced JNK activity, but also protected mice from cerebral ischemia-hypoxia that was attributed in part to a reduced expression of *Bim* and *Fas* (Kuan et al. 2003). Recently, the specific activation of the PI3K/Akt pathway has been identified as an additional neuroprotective mechanism by gene expression profiling in *jnk3* knockout mice (Junyent et al. 2011). Novel data further extend the understanding of these processes by demonstrating that in principle all JNKs can induce cell death, which is critically dependent on nuclear localization and activation of cell death pathways (Bjorkblom et al. 2008).

In the context of PD the JNK/c-Jun pathway and especially the JNK3 isoform seem to play a leading role: in a human autopsy study c-Jun was found activated in DAergic neurons from PD patients—a result that was equally observed in the MPTP mouse model of PD (Hunot et al. 2004). In the same study, an examination of various JNK-deficient mice showed that both JNK2 and JNK3, but not JNK1, were required for MPTP-induced c-Jun activation and DAergic cell demise. Mice deficient for JNK3 showed also an enhanced and lasting survival of the MFB axotomized DAergic neurons in the SNpc (Brecht et al. 2005). JNK3 was also shown to mediate paraquat- and rotenone-induced DAergic neuron death, which could be attenuated by pharmacologically blocking translocation and activation of JNK3 (Choi et al. 2010). However, low JNK3 levels were not able to attenuate striatal dopamine terminal loss (Pan et al. 2009). In vivo studies in JNK3 or JNK2/3 knockout mice were not able to demonstrate axonal regenerative effects in a model of glaucoma (Quigley et al. 2011) or in an experimental model of PD (Cheng et al.

2010), which may be due to the more complex in vivo situation involving compensatory mechanisms in these genetically modified mice. Induced models to interfere with JNK3 signaling could possibly shed more light on the situation in vivo.

To this end, specific small molecule inhibitors of the JNK isoforms including JNK3 have been recently developed (Bowers et al. 2011; He et al. 2011; Probst et al. 2011; Schattel et al. 2011) and could be complementary to gene therapeutic approaches using siRNA/shRNA. Nevertheless, even with improved cell permeability of such JNK3 inhibitors, the achievement of a balanced specific spatial and temporal cellular inhibitory activity in vivo will still be required (Antoniou et al. 2011).

Studies on autopsy cases from patients treated with intraputamin GDNF injections impressively demonstrate that regeneration can take place in PD (Love et al. 2005). Therefore, there is evidence to consider axonal regeneration as a therapeutic target mechanism in addition to neuroprotection in PD.

Taken together, we have shown that inhibition of JNK3 may result in a detrimental outcome regarding survival of previously lesioned and subsequently regenerating DAergic neurons. In our paradigm, JNK3 appears to be required for both axonal regeneration and survival. Thus, our results strongly support a pro-regenerative role of JNK3 and attribute a pro-survival role specifically for lesioned dopaminergic neurons.

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